DECLARATION BY DR. MURIEL MOSER

I, Muriel Moser, declare as follows:

I hold a Ph.D. degree in Zoology with greatest distinction from the Free University of Brussels, Belgium. Specialized in zoology since 1977, I am heading a research group focusing on the physiology of antigen presenting cells (since 1986) at the free University of Brussels. I hold, since April 2002, a degree of "Agrégé de l'enseignement supérieur" I frequently lecture at international meetings and I am a regular reviewer for several international Journals. I author over one independent, world-wide patent application and over 50 international peer-reviewed publications in the field of cell-based immunology. I have initiated the study of murine dendritic cells at the free university of Brussels and I am experimenting since the filing date of the present patent application onwards successfully with the production of human DC/tumor hybrids/hybridomas and their use to eliminate cancer in patients. I am the past president of the French group on dendritic cells. I was appointed several times as the European expert on Immunology. I enclose my Curriculum Vitae in annex (Enclosure 1).

I am one of the inventors of the US patent application US 09/802,397 and have reviewed and understand all prior art and Office Actions of record. The present Declaration illustrates that the present invention relies on unexpected results, therefore this declaration may be helpful for the examiner in advancing prosecution.

1. The claimed subject-matter

The present invention relates to a method of producing an anti-tumor response in a mammalian subject, said method comprising administering to said subject a plurality of dendritic cell/tumor hybrids and/or a dendritic cell/tumor hybridoma. The present invention illustrates for the first time that said cells may be produced and carry characteristics of tumor cells and DCs which makes them interesting for cancer therapy.

Furthermore, the present invention proves for the first time that tumors are efficiently eliminated using said hybrids/hybridomas. In particular, I have shown in this present application that said approach may easily be followed, efficient and applicable in humans.

Before the filing of the applications whereto the above-mentioned patent application claims priority (US 09/049,502; 09/025,405; 08/625,507 and 08/414,480) nobody gave the experimental/clear proof for the generation of dendritic cells (DC)/tumor hybrids or hybridomas. In particular, nobody described hybrids/hybridomas which may be applied in animal, especially human, therapy.

The present invention further teaches that DC/tumor hybrids/hybridomas may be produced efficiently starting from proliferating dendritic cells or a dendritic cell at a more immature stage. Until now, there is no cellular characteristic or marker available which may be used to define this preferred DC-fusion partner. The only definition which may apply is that said cells may be proliferating DC or are DCs at a more immature stage.

Furthermore, in order to make DC/tumor hybrids allowable in human medicine, it is essential that no essential body-part(s) of the patient is(are) used (such as spleen). A

solution to this problem is also formulated in the present invention. The present invention suggests to use cells found in for instance bone-marrow, lymph/lymph nodes or in blood. According to the present invention said cells may be proliferating dendritic cells or a dendritic cell at a more immature stage as mentioned above. Said dendritic cells may originate from induced DC-progenitors.

The approach to make human DC/tumor hybrids/hybridomas, which is applicable in human medicine, has never been suggested before the filing of any of the US applications to which the present US patent application claims priority to. Based on the prior art it was not predictable that by using dendritic cells, or, proliferating dendritic cells or a dendritic cell at a more immature stage, as described in the present application, hybrids/hybridomas could be made having both the DC and tumor characteristics which are needed to trigger tumor elimination in a patient.

2. Non-obviousness of the subject-matter of claims 1 and 3 over Guo et al. (1994) in view of Somasse et al. (1992)

I respectfully disagree with the assertion in the outstanding Office Action that that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute the dendritic cells taught by Somasse et al. (1992) for the B cells of Guo et al. (1994) as the APC fusion partner in producing a dendritic cell/tumor cell hybrid, and, that it would be obvious to administer said product to a subject for production of an anti-tumor response.

In my opinion, based on said documents it is not predictable that such hybrids could be made and which characteristics said hybrids would carry. Furthermore, I am convinced that the approach of Guo for making his hybrids may not be followed to produce hybrids which may be used for human applications. As the method of making said hybrids (and thus also the starting cells) are different, it is clear that the resulting hybrids will be different. These different aspects are discussed in the paragraphs below.

The difference between the present invention and the teachings of Guo and Somasse lays thus in the definition of the hybrids AND in their use.

a/ The feasibility of making DC/tumor hybrids is not predictable

Changing the fusion partner of the tumor cell, as with the B cell of Guo et al. (1994), to another antigen-presenting cell does not allow one of skill in the art to predict the outcome of such an experiment. As mentioned in the discussion section of Carbone et al. (1988, see copy in annex (Enclosure 2), p 1374, first column, second paragraph, l.10-12), extinction or loss of expression of tissue specific traits after fusion of dissimilar cells is a well-established phenomenon (Lewin 1980; Killary and Fournier 1984). This negative regulation is not specific for cell fusions but was also observed for incoming genes (Clough et al. 1982; Palmiter et al. 1982; Gautch and Wilson 1983; Groffen et al. 1983; Hardies et al. 1983; Manor 1985; Humphries et al. 1985; Dyson et al. 1985) (p 1374, second column, second paragraph, l.1-7 of Carbone, et al.). Methylation has been shown to be a commonly used system to regulate expression within the cell; it has even been shown to be used for the natural regulation of certain T-cell surface molecules (Richardson et al. 1986) (p 1374, first column, second paragraph, l.10-15 of Carbone, et al.).

Only after extensive experiments carried out by my group it was possible to conclude that fusing tumor cells with dendritic cells as described in the present application can result in the generation of hybrids/hybridomas that carry tumor antigenic markers combined with various dendritic specific markers. The success of this fusion could not have been predicted by the prior art, and in fact, there would be sufficient reasons for one of ordinary skill in the art to believe that such a fusion of two dissimilar cells would not work, based on the above-described phenomenon of loss of expression of tissue specific traits after fusion. One of ordinary skill in the art would have taken a very cautious attitude and would not have predicted a successful outcome for this experiment until it had been demonstrated.

In this respect, it should be recognized by the Examiner that experimental evidence for the possibility to produce an anti-tumor response in a subject comprising administering dendritic cell/tumor cell hybrids/hybridomas was given for the first time in the parent of the present U.S. patent application. To reason that the outcome of the present experiments would have been predictable in advance or would be obvious is only possible when one reasons with hindsight. It is clear from the reasons cited above that the skilled person would not have reasoned in this way and would not have predicted any outcome of such an experiment before it had been proven that it works.

I hereby respectfully submit that it was not *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute the dendritic cells taught by Somasse et al. (1992) for the B cells of Guo et al. (1994) as the APC fusion partner in producing a dendritic cell/tumor cell hybrid. Therefore I respectfully submit that the administration of the hybrids/hybridomas of the present invention to a patient in order to produce an anti-tumor response in said patient is not obvious over Guo et al. (1994) in view of Somasse et al. (1992).

b/ The approach of Guo cannot be followed to produce hybrids/hybridomas for in vivo treatments of animals (humans)

Further, in support of the inventive step of especially claims 1 and 3, I wish to point towards the impossibility to use the method used in Guo et al. (1994) for the production of hybrid cells for animal (especially human) applications.

The method according to Guo et al. (1994) involves the use of B cells as fusion partners of the tumor cells. Said B-cells were recovered from the spleen of rats earlier injected with with soluble antigen in complete Freund's adjuvant, which cannot be applied in humans. In addition, if immunizations are done without Freund's adjuvant, the outcome of the B cells remains unpredictable in individual animals and it is expected to be unpredictable in individual human patients.

Furthermore, the approach followed by Guo does not allow multiple booster applications. In this respect I wish to stress the difference between the method used to generate hybridomas and the method used in Guo et al. (1994). In the present invention hybridomas are selected by growing them in selection medium. Unfused immortalized cell lines are killed by the exposure to a drug. In the description of the present application the use of the HAT (hypoxanthine-aminopterin-thymidine) selection medium is illustrated. After selection, the hybridoma can be cultured when needed and used for multiple booster vaccinations. This is a major advantage compared to the strategy used by Guo et al. (1994) where fusions are made and immediately used for treatment without making them immortalized;

each treatment needs a separate fusion step where variability in cell population between booster applications can be generated.

A further advantage of the DC/tumor hybrids/hybridomas of the present invention over the hybridomas of Guo et al. is that no essential body parts are needed as start material, that the fusion partners are easy to isolate, that it allows the treatment of a subject using cells which are compatible with its immune system and that the approach of the present invention allows to produce a product for which a guarantee of its composition and quality may be given. All these aspects are discussed in detail in section 3 (see below).

3. Non-obviousness of the subject-matter of claims 19-26 over Guo et al. (1994) in view of Sornasse et al. (1992)

I am of the opinion that the note of the Examining Division in respect of the fact that the spleen cells of Guo et al. (1994) and Sornasse et al. (1992) would also comprise an isolated DC as well as the only two known murine subtypes of DC (i.e. myeloid and lymphoid, both of which are derived from bone marrow) is inappropriate.

I would like to point out that both myeloid and lymphoid DC may be considered as isolated DCs.

Claims 19-20 and 47-50

It is true that all DCs found *in vivo* (thus also from spleen) are originally bone marrow derived. However, what the present application teaches is different. The present application illustrates that DC-cells derived from bone marrow, lymph/lymph nodes, blood or other tissues are a better alternative to spleen cells to start the production of the hybrids of the present invention.

I confirm hereby that my group has the experience that a DC-preparation from spleen is not a good start population to aim for the production of DC/tumor hybrids/hybridomas. Indeed I have the experience that primary cultured DCs (proliferating DCs) are preferentially needed (e.g. cultured from bone marrow cells) to produce said hybridomas; using primary DCs (non-proliferating DCs; e.g.isolated from spleen) will not result in the efficient and reproducible production of DC/tumor hybrids/hybridomas. I have the experience that when using preparations of spleen cells mainly hybrids may be formed between non-DCs (for example T cells) and tumor cells even if the DC population in said preparation is dominant. Consequently, I am of the opinion that starting from mouse splenic cells is extremely doubtful that a real DC/tumor hybridoma can be obtained.

In addition, bone narrow, lymph/lymph nodes and blood may contain DC-precursors or intermediates between monocytes and DCs. Said monocytes may be used to further differentiate into proliferating intermediates between monocytes and DCs or into proliferating DCs before the production of the hybrids/hybridomas of the present invention. These intermediates or precursor cells are not present in tissues such as spleen.

In the section below I further explain that the method applied by Guo to produce the DC/tumor hybridomas may not be applied in medicine (humans or animals) and thus has no industrial value.

A further optimization of DC/tumor hybrids of the present invention, is that said hybrids/hybridomas may be applied in medicine (human and animal). Indeed, the DC used to make the hybrids/hybridomas of the present inventi n are purified from cells without the need for isolating essential organs/cells from said mammals such as spleen. Using spleen cells, as used in the method of Guo et al, 1994, inherently results in the killing of the organism from which said cells are retrieved. Contrarily, the use of DC fusion partners derived from blood, lymph/lymph nodes or bone marrow as proposed in the present invention allows to keep the organism, from which said cells are isolated, alive.

Furthermore, a patient may be treated with cells derived fom his own body making the cancer treatment compatible with his immune system. A higher efficiency of the therapy is therefore also expected.

In summary, the use of DC-fusion partners purified from blood, lymph/lymph node or bone marrow cells allows a more efficient production of the hybrids/hybridomas of the present invention. In addition, these bone marrow and blood cells are easy to isolate and allow a better approach for human applications. I also refer to the arguments given in section 2/b (above).

Claims 21-26

I hereby explain the cellular origin of existing DCs in animals.

DC precursor cells from bone marrow may be considered as the stem cell from which DC myeloid precursor cells (e.g. monocytes) and DC lymphoid precursor cells may differentiate. Both precursor cells may give rise to differentiated, also called mature DCs; myeloid and lymphoid DC respectively.

Mature myeloid and lymphoid DCs may be found in specific tissues, such as spleen, but may also be present in for instance blood. Precursor cells of myeloid or lymphoid origin may be present all over the body. However, it is accepted in the scientific literature that these are not present in specific tissues, such as spleen.

Most stem cells (bone marrow) or precursor cells are present in a resting state. This means that these are non-proliferating. However, proliferation may be induced (in vivo or in vitro) in said cells.

I have the experience that the hybrids/hybridomas of the present invention may be made with high efficiency when differentiating blood, lymph/lymph nodes or bone marrow DC-precursors or proliferating DCs (before they enter in the resting state) are used. Said cells are therefore considered as preferable fusion partner for the production of the hybrids/hybridomas of the present invention (new claims 29-46).

4. Non-obviousness of the subject-matter of claims 5-10 over Guo et al, (1994) in view of Sornasse et al, (1992) and in further view of U.S. Patent 5,851,756.

As explained above, skilled person may not derive from Guo et al. and Sornasse that such a DC/tumor hybrid may be formed.

In addition, I respectfully disagree that based on the '756 patent one f ordinary skill in the art at the time of the invention would have been motivated to induce DC-characteristics (using GM-CSF) in DC/tumor hybrids/hybridomas before using said fused cells in anti-tumor treatments.

This is non-obvious based on the following reasons:

1/ The induction in the method of the present invention is performed *after* the hybrid formation, the DCs corresponding to the DCs of the '756 patent do not exist anymore.

2/ The DCs of the '756 patent are different from the hybrids of the present invention. It is not obvious that such a hybrid would behave like the isolated DC cell of the '756 patent.

3/ The induction allows to induce the expression of DC characteristics, not to increase the number of DC cells in blood as taught in the '756 patent and explicitly repeated by the Examining Division.

5. Non-obviousness of the subject-matter of claims 11-14 over Guo et al. (1994) in view of Somasse et al. (1992) and in further view of U.S. Patent 5,637,483.

I respectfully disagree that based on the '483 patent an ordinary skilled in the art at the time of the invention would have been motivated to irradiate the hybrids/hybridomas of the present invention to prevent proliferation.

According to the '483 patent, the irradiation of the tumor vaccine is presented as an essential step. I hereby refer to the claims of said patent and to especially example 6 of said patent. In said example, vaccination studies using life transduced tumor cells are discussed. In said experiment (column 14, l.48-50) it is explicitly mentioned that all tumor cells, except the IL-2 secreting tumor cells, resulted in tumor formation. It is obvious that life-tumor-cells would never be accepted as vaccine in human therapy.

Contrarily, in the present invention it is suggested that said hybrids/hybridomas may be irradiated. However, said irradiation is not an essential step in the production of said vaccine. Indeed, the hybrids/hybridomas of the present invention are fusions between tumor cells and DC cells. They may have predominant tumor characteristics, predominant DC characteristics, or an equal distribution of both characteristics. For cells with especially predominant DC characteristics, said irradiation may have a more negative effect on the anti-cancer therapy. The present invention teaches explicitly that also living hybrids/hybridomas elicited an anti-tumor immune response (paragraph [0100], l.9-12 of the application as published). Therefore, said cells have predominantly DC characteristics and do not need the irradiation step as proposed in the '483 patent before it is used in the anti-cancer therapy.

Said irradiation should be considered as an essential step in the approach of the '483 patent, and is optional in the method of the present invention. This proves that the hybrids of the present invention are clearly different from the tumor cells of the '483 patent, and that the characteristics of said hybrids are not predictable based on the tumor cells of the '483 patent.

I am convinced that the teachings of the present invention and the '756 patent are not comparable.

Declaration Dr. Moser
US Patent Application No. 09/802,397

I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true.

Signed this 7 day of November, 2003

Muriel Moser